



## Histopathology of the salivary glands of the cockroach, *Periplaneta americana* (Blattodea: Blattidae) induced with insecticide cypermethrin

Sohidul Islam<sup>1</sup>, Mohammad Amir<sup>1\*</sup>, Shagufta Yasmeen<sup>1</sup>, Beenish Mirza<sup>1</sup>, Faizan<sup>2</sup>

<sup>1</sup> Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

<sup>2</sup> Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India

### Abstract

The present study aimed to investigate the effect of a synthetic pyrethroid insecticide, cypermethrin, on the salivary glands of the American cockroach, *Periplaneta americana*. Insecticide cypermethrin of the concentrations 0.0005% and 0.005% was provided in feed to adult cockroaches to examine the alterations from normal histology of salivary glands after treatment. Results indicated that salivary glands were highly affected after 24 hours post-treatment. The changes observed in the cypermethrin-treated salivary gland were categorized by alteration in shape and size, degeneration and swelling of central and peripheral cells, elongation of duct epithelial cells, and contraction of duct lumen as compared to the control group. The damage caused by cypermethrin in the salivary gland acini and duct developed more extensively as the concentration of cypermethrin increased.

**Keywords:** cockroach, histopathology, *Periplaneta americana*, salivary glands

### Introduction

*Periplaneta americana* belongs to the family Blattidae of the order Blattodea and is commonly known as the American cockroach. Geographically, *P. americana* is distributed worldwide but is native to Africa and the Middle East. In India also, they are widely dispersed. This cockroach species is generally found outdoors in vegetation, under mulch, and in homes [1]. They are reddish brown and are about 3 inches long. They are omnivorous and feed on various foodstuffs, including bread, fruit, feces, dead insects, etc., leaving an unpleasant odor. *P. americana* causes a significant annoyance in homes. Also, it acts as a carrier of various bacteria, e.g., *Salmonella*, *Shigella* and, *Cryptosporidium parvum*, *Klebsiella pneumoniae*, which cause diarrhea, pneumonia, and nosocomial infections [2, 3]. Cockroaches also act as mechanical vectors in transmitting typhoid, dysentery, and leprosy causative agents. Substances produced by cockroach cause allergies in humans [4, 5]. These allergens are also associated with asthma. Also, secretions of cockroaches (Xanthurenic acid+kiturenic acid+8-hydroxycuinalic acid) have mutagenic and carcinogenic properties [6].

An alimentary canal and its associated glands comprise the digestive system of cockroaches. In front of the mouth, a preoral cavity is surrounded by mouthparts. The mouth is divided into two chambers by the hypopharynx. The anterior chamber of the mouth is named as cibarium, and the posterior one is called the salivarium. The alimentary canal of the cockroach extends from the mouth to the anus and is divided into foregut or stomodaeum, midgut or mesenteron, and hindgut or proctodaeum. Their alimentary canal is in the form of a long tube and is coiled in some places. Salivary glands of cockroaches have paired structures and are white in colour. These have a glandular portion and a hollow receptacle or reservoir, and they are located on either side of the oesophagus in the prothoracic region. Each of the compressed glands' tiny, duct-like openings is lined with hexagonal glandular cells. The receptacle is positioned

between two pairs of glands on either side and is kept connected by a duct. Two glands' ducts on one side combine to form a single salivary duct, and the salivary ducts from two sides combine to form a common duct. After receiving the ducts from two receptacles, the common duct merges into a spacious efferent salivary duct. The base of the hypopharynx, located on the floor of the buccal cavity, is where the efferent salivary duct opens. Digestive enzymes are secreted by the salivary glands and kept in reservoirs until needed to break down the ingested food. Using optical, electron, and confocal laser scanning microscopy, Just and Walz (1994a) [7] studied the morphology of the salivary glands of the cockroach *P. americana* [7]. They showed that every secretory acinus has a uniform structural design. They comprise three different cell types: duct, central, and peripheral. Each acinus' base comprises a single pair of peripheral cells. The central cells are located downstream and are grouped concentrically in a fourfold symmetry around the acinar ducts' most proximal region. Duct cells surround the central cells in every acinus, forming a thin sheet on their apical surface. The central cells only pass the contents of their secretory vesicles through these holes into the duct lumen because the apical lining of the duct cells is frequently fenestrated. Their analysis of ultrastructural data supports the idea that only central cells secrete the proteinaceous components of saliva, while the peripheral cells are in charge of transporting water and electrolytes. These researchers have discovered that the duct cells distal to the acini are particularly trained in transporting ions and water.

Various methods have been explored to control this largest species of common cockroach. Numerous garden herbs, including catnip, bay, mint, cucumber, and garlic, have been employed as repellents. Baits made with powdered boric acid and fipronil have also been successful. Since cockroaches are resilient and fast-breeding, chemical insecticides are needed to control their population. Some scientists have found deltamethrin effective against

cockroaches. A synthetic pyrethroid insecticide called cypermethrin is also employed in consumer goods for residential usage and in large-scale commercial agriculture purposes. In insects, it functions as a neurotoxin. They work by interfering with the voltage-gated sodium channel's (VGSC) regular operation. Cypermethrin is quickly broken down by soil and plants, but it retains its effectiveness for several weeks when applied on inert indoor surfaces. Cypermethrin is also a component in ant and cockroach killers, including Raid, Ortho, Combat, and ant chalk.

Here the present study deals with the different concentrations (0.0005% and 0.005%) of cypermethrin to observe its histopathological effects on the salivary glands of American Cockroach, i.e., *P. americana*. In comparison with normal histology of the salivary glands, using the insecticide cypermethrin in the experiment that aims to find any change or an alteration in the cells of the glandular tissues of the *P. americana* with a particular observation in the cells of acini, duct cells, deterioration in cells, changes in cell shape and size, cytoplasmic vacuolization, the fate of nucleus, etc. Using the different concentrations (0.0005% and 0.005%) of the insecticide cypermethrin will help understanding its chemical effects on the target sites of the salivary gland as stated above. The experiment may further determine the efficacy of cypermethrin as an insecticidal chemical agent for combating cockroaches.

#### Materials and methods

*P. americana* adults were gathered from sewage maintenance openings in and around the Aligarh Muslim University campus in Aligarh, India, and brought to the lab. These insects were cultivated at room temperature and housed in rearing jars. Breadcrumbs were served as food for the insects and were replaced every other day. To maintain the insect's appropriate culture, overcrowding was avoided.

#### Sampling of experimental insects

Adults received varied cypermethrin concentrations after being kept in a separate container. In feed (bread crumbs), 2 ml of each concentration (0.005% and 0.0005%) were administered. The treated insects were then kept under close watch.

#### Application of insecticide

A uniform mixture of feed and 2 ml of each insecticide concentration (0.005% and 0.0005%) were placed in each Petri dish which was kept in jars 1 and 2 separately, and five cockroaches were released in each jar. In addition to these, the control setup was also observed. After 24 hours, cockroaches were dissected in insect Ringer's solution to collect salivary glands for later research.

#### Preservation and histological preparation

The cockroaches were dissected after 24 hours of examination. Samples for histopathological study were prepared according to the procedure of Yasmeen and Amir (2018) [8]. The salivary gland was excised and was immediately fixed in Bouin's solution (18-20 hours). To remove the surplus fixative, washing was done twice or three times in tap water. Dehydration then continued with increasing levels of alcohol, such as 30%, 50%, 70%, 80%, and 90% for 5 minutes each, 96% and 100% for 30 minutes each, then a mixture of 100% and xylene solution (1:1) for 10 minutes. After that, the tissue was then incubated in

xylene and paraffin (1:1) at 60 °C for 15 minutes, after which it was incubated in pure wax for 2 hours. The salivary gland's small lobules were encased with paraffin wax. After a day, a microtome was used to cut 6 µm sections from each prepared block. The ribbons were then put on the glass slide greased with an albumin and glycerine (1:1) solution. The section-containing slides were lightly warmed to remove any wrinkles.

The slides were stained by running them through two cycles of xylene, followed by five minutes in each of the following descending alcohol concentrations: 100%, 96%, 90%, 80%, 70%, 50%, and 30%, followed by five minutes in distilled water. Slides were stained in Delafield's hematoxylin for 10 seconds, maintained in tap and distilled water for 5 minutes each, and counterstained with eosin for 20 minutes. After that, slides were dehydrated in ascending grades of alcohol, followed by two changes of xylene for 5 minutes each, then stained again with two cycles of xylene for 5 minutes each. Slides were mounted in DPX and examined under a microscope after air drying. Photomicrograph was taken using a LEICA DFC 295 microscope using 100X and 400X magnification.

#### Results

##### Normal histology of salivary gland of *P. Americana* (Fig. 1a&b)

Each side of the oesophagus and crop is lined by an enormous salivary gland and its corresponding reservoir in *P. americana*. The gland is a thin, lobulated mass with two principal lobes that resemble leaves. The salivary glands of *P. americana* are a branched duct system; the smallest ducts terminate in spherical glandular acini, and the large main ducts join the main duct of the reservoirs separately.

The Light microscopy showed that the outer surface of each lobe of the salivary glands was relatively smooth, and a thin basal lamina enveloped all salivary gland units. Under the basal lamina, the plasma membrane of cells is highly invaginated, forming deep basal membrane infoldings in the cells. Every acinus comprises a fibrous sheath enclosing two main cell types- peripheral and central cells with well-developed nuclei. The bulk of each acinus is occupied by large central cells. The basal surface of this cell occupies a relatively minor fraction of the acinar surface, and it is not extensively infolded. In contrast, the major part of the periphery is occupied by numerous small peripheral cells. The peripheral cells are pyramidal, and their apical plasma membrane is deeply infolded. The salivary ducts are composed of cuboidal epithelial duct cells with small nuclei. The lumen of the duct is lined with a layer of chitin, which is electron opaque.

##### 1. Histopathological effects of cypermethrin on the salivary gland of *P. americana*

###### 0.0005% Concentration of cypermethrin (Fig. 1c &d)

Cells of acini get deformed in shape. Central and peripheral cells in acini became swollen, and cell deterioration was noticed. Cytoplasmic vacuolization increased in cells of acini. Nucleus became vanished in some acinus cells.

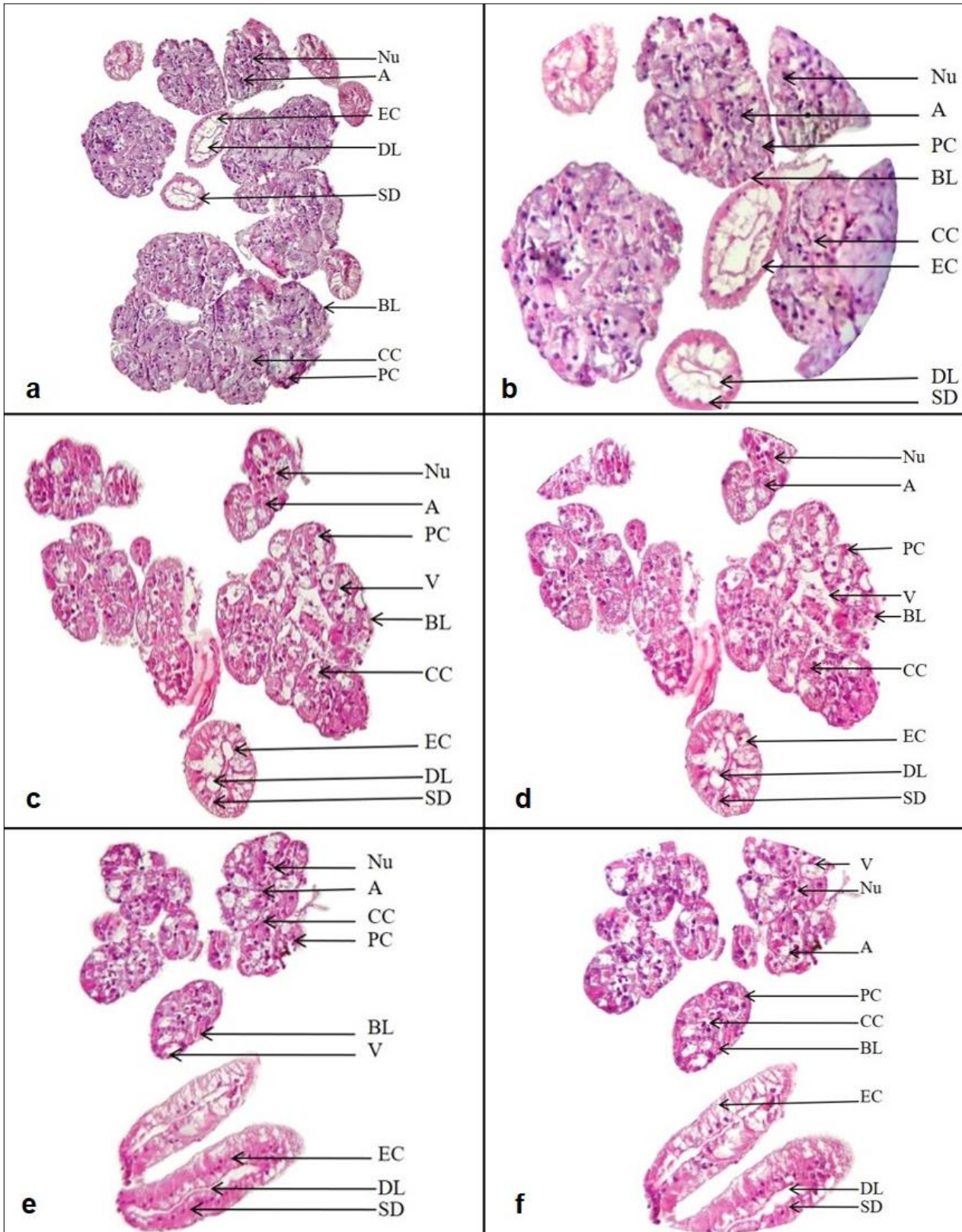
In the case of the salivary duct, epithelial cells got elongated and were ruptured. Reduction of cytoplasm in duct epithelial cells was noticed. No effect on the shape of the salivary duct was seen, but the lumen of the duct decreased slightly.

**2. 0.005% Concentration of Cypermethrin (Fig. 1e&f)**

Acini cells get more deformed as compared to 0.0005% concentration. The swelling of the central and peripheral cells was more pronounced compared to the lower concentration. Also, the deterioration, reduction of cytoplasm, and cytoplasmic vacuolization of central and peripheral cells were more conspicuous in this

concentration. Nucleus gets lost from some acinus cells.

At this concentration, the effect on the salivary duct was more prominent. The shape of the salivary duct became highly elongated, and the lumen of the duct was extremely contracted. Duct epithelial cells were disrupted with a reduction of cytoplasm. Nucleus became invisible in a few duct cells.



**Fig 1:** Transverse section of salivary gland of *Periplaneta americana*, control (a) 100X, (b) 400X; treated with 0.0005% cypermethrin after 24h (c) 100X, (d) 400X; treated with 0.005% cypermethrin after 24h (e) 100X, (f) 400X. [A- acinus, BL- basal lamina, CC- central cell, DL- duct lumen, EC- epithelial cell, Nu- nucleus, PC- peripheral cell, SD- salivary duct, V- vacuole]

## Discussion

The current study demonstrates that adult *P. americana* salivary glands experience cypermethrin-induced histological changes. When compared to the control group, the cypermethrin-treated *P. americana* showed altered size and shape, degeneration and swelling of central and peripheral cells, elongation of duct epithelial cells, and contraction of duct lumen. As the concentration of cypermethrin rose, more extensive cypermethrin damage was seen in the salivary gland acini and duct.

Over 30% of insecticides are synthetic pyrethroids, which are thought to be the least toxic [9]. Due to their quick biodegradability and target-oriented mechanism of action, these insecticides are preferred [10, 11]. A typical synthetic pyrethroid used in veterinary medicine, agriculture and household insect/pest management is cypermethrin [12]. It debuted on the market in 1977. The chemical structures of all the insecticides in this class are roughly based on pyrethrins, which are insecticidal substances found in *Chrysanthemum* flowers. Cypermethrin is a complex chemical, like the majority of synthetic pyrethroids. All eight isomers can be found in cypermethrin. Over 90% of the cypermethrin produced globally is used to eradicate insects on cotton. Cockroaches (and other indoor pests) and termites are also killed using it on lettuce and pecans. Humans exposed to cypermethrin by skin contact or food are only mildly harmful. By prolonging the transient increase in sodium permeability of neuronal membrane channels during excitation, cypermethrin induces neurotoxicity in mammals and insects. There may be hundreds to thousands of recurrent nerve impulses brought on by these long-lasting trains in the sense organs. The voltage-dependent sodium channel is damaged due to the pyrethroid's repeated action, which prolongs the time sodium channels are open [13]. The neurological system is also affected by cypermethrin in different ways. Excitation and convulsions are brought on by inhibiting the gamma-aminobutyric acid receptor. Additionally, it prevents monoamine oxidase, an enzyme that degrades neurotransmitters, and nerve calcium uptake. Adenosine triphosphatase, an enzyme not directly connected to the nervous system, is likewise impacted by cypermethrin. It is involved in the synthesis of cellular energy, the movement of metal atoms, and the contraction of muscles.

The current investigation showed that, compared to the control group, cockroaches exposed to 0.0005% of cypermethrin had histologically changed salivary gland cells, a change in the cell structure, and cytoplasm vacuolation. This outcome is consistent with the findings of Nodari *et al.* (2011) [14], who reported the first signs of glandular tissue disintegration such as the presence of vacuolation in the cytoplasm and, more importantly, in the nucleus of the acinar cell, as well as changes in the form of the cell (irregular in shape and increase in size), when the salivary gland of female ticks was treated with a concentration of 206 ppm of permethrin.

Changes in the salivary gland cells of treated adult *P. americana* became more pronounced as the cypermethrin insecticide concentration rose (0.005%), indicating that this concentration would lead to a more significant destruction of treated adult *P. americana* salivary gland cells. The gland cell membrane ruptured and glandular cell disintegration was brought on by this dose. Additionally, central and peripheral cells' degeneration, loss of cytoplasm, and

cytoplasmic vacuolization were more obvious in this concentration. Some acinus cells lose their nuclei. These findings were consistent with those of Furqum *et al.* (2008) [15], who reported that just a few acini cells were detected in ticks exposed to 2060 ppm of permethrin. They eventually lost their histological and morphological characteristics due to disintegration, the acini cells' membrane losing its integrity, the cytoplasm vacuolizing, and nuclear size and shape changes.

As food is swallowed, the salivary gland secretion moistens, dissolves, and partially digests it. According to Just and Walz (1994a-c) [7] and Kessel and Beams (1963) [18], central cells are responsible for producing and secreting proteins and mucins, while peripheral cells are focused on transporting electrolytes and water [7, 16, 18]. The production and release of proteins and mucins in the salivary gland of *P. americana* may have been compromised due to changes in these cells following insecticide treatment.

Thus, the current study demonstrated that cypermethrin has insecticidal properties that directly affected the salivary gland of adult *P. americana*, which was crucial in drawing conclusions from the results and providing a basis for future research into the effectiveness of cypermethrin as a potential agent for cockroach control.

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